

a-d, i, q-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = polymer.

FIG. 39A

```
CHO, BHK, 293 cells, Vero expressed Cerezyme a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.
```

- 1. Sialidase
- 2. CMP-SA-PEG (16 mol eq), ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.
```

## FIG. 39B

```
CHO, BHK, 293 cells, Vero expressed Cerezyme. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.
```

- 1. Sialidase
- 2. CMP-SA-M-6-P (1.2 mol eq), ST3Gal3
- 3. CMP-SA (16 mol eq), ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = mannose-6-phosphate
```

### FIG. 39C

```
CHO, BHK, 293 cells, Vero expressed Cerezyme. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.
```

- 1. Sialidase
- 2. CMP-SA-PEG (16 mol eq), ST3Gal3
- 3. CMP-SA, ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = Mannose-6-phosphate
```

# FIG. 39D

```
CHO, BHK, 293 cells, Vero expressed Cerezyme. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.
```

- 1. CMP-SA-levulinate, ST3Gal3, buffer, salt
- 2. H<sub>4</sub>N<sub>2</sub>-spacer-M-6-P or clustered M-6-P

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = M-6-P or clustered M-6-P
```

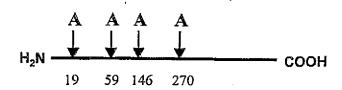
FIG. 39E

CHO, BHK, 293 cells, Vero expressed Cerezyme. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

1. CMP-SA, α2,8-ST

a-d, i, q-u (independently selected) = 0 or 1; e-h = 1; j-m (independently selected) = 0-20; v-y (independently selected) = 0.

FIG. 39F



$$A \leftarrow \begin{array}{c} \\ \text{(Fuc)}_{i} \\ \text{GlcNAc-Man} \\ \text{(GlcNAc-(Gal)}_{a}]_{e} - (Sia)_{j} - (R)_{v} \\ \text{[GlcNAc-(Gal)}_{b}]_{f} - (Sia)_{k} - (R)_{w} \\ \text{[GlcNAc-(Gal)}_{c}]_{g} - (Sia)_{l} - (R)_{x} \\ \text{(R')}_{n} \\ \text{[GlcNAc-(Gal)}_{d}]_{h} - (Sia)_{m} - (R)_{y} \\ \text{[GlcNAc-(Gal)}_{d}]_{h} - (R$$

a-d, i, n, p-u (independently selected) = 0 or 1.
e-h (independently selected) = 0 to 6.
j-m (independently selected) = 0 to 100.
v-y = 0;
R = modifying group, mannose, oligo-mannose;
R' = H, glycosyl residue, modifying group,
glycoconjugate.

FIG. 39G

```
Insect cell expressed Cerezyme.
a-d, f, h, j-m, s, u, v-y = 0;
e, g, i, q, r, t (independently selected) = 0 or 1.
```

- 1. GNT's 1,2,4,5, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal-PEG

```
a-i, q-u (independently selected) = 0 or 1;
j-m = 0;
v-y (independently selected) = 1,
when e-h (independently selected) is 1;
R = PEG.
```

## FIG. 39H

```
Yeast expressed Cerezyme.

a-m = 0; q-y (independently selected) = 0 to 1;

p = 1; R (branched or linear) = Man, oligomannose.
```

- 1. Endoglycanase
- 2. Galactosyltransferase, UDP-Gal

a-m, p-y = 0; n (independently selected) = 0 or 1; R' = -Gal-Sia-PEG.

## FIG. 391

```
CHO, BHK, 293 cells, Vero expressed Cerezyme. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.
```

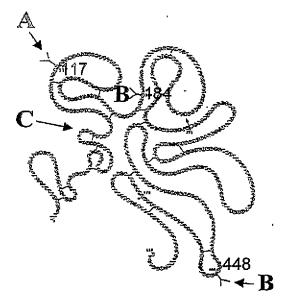
- 1. CMP-SA-linker-SA-CMP, ST3Gal3
- 2. ST3Gal3, desialylated transferrin.
- 3. CMP-SA, ST3Gal3

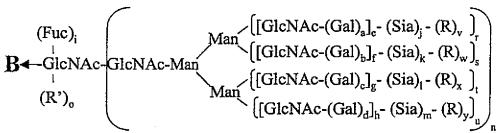
```
a-m, q-u (independently selected) = 0 or 1;

p = 1; n = 0; v-y (independently selected) = 0 or 1;

R = linker-transferrin.
```

FIG. 39J





$$\mathbf{C} \leftarrow \text{-(Fuc)}_{0-1}$$
 $\mathbf{A} \leftarrow \text{-GlcNAc-GlcNAc-Man}$ 
 $\mathbf{Man} - [\text{Man}]_{0-12}$ 
 $[\text{Man}]_{0-6}$ 
 $[\text{Man}]_{0-6}$ 

a-d, i, n-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 4. j-m (independently selected) = 0 to 20. R = polymer; R' = sugar, glycoconjugate.

FIG. 40A

```
CHO, BHK, 293 cells, Vero expressed tPA a-g, n = 1; h = 1 to 3; j-m, i, (independently selected) = 0 or 1; r-u (independently selected) = 0 to 1; o, v-y = 0.
```

- 1. Mannosidase(s), sialidase
- 2. GNT1,2 (4 and/or 5) UDP-GlcNAc
- 3. Gal transferase, UDP-Gal
- 4. CMP-SA-PEG, ST3Gal3

```
A = B; a-g, n = 1; h = 1 to 3;
i, r-u (independently selected) = 0 or 1;
o = 0; j-m, v-y (independently selected) = 0 or 1;
R = PEG
```

### FIG. 40B

```
Insect or fungi cell expressed tPA
A = B; a-d, f, h, j-o, s, u, v-y = 0;
e, g, i, n, r, t (independently selected) = 0 or 1.
```

- 1. GNT's 1&2, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal
- **★** 3. CMP-SA-PEG, ST3Gal3

```
A = B; b, d, f, h, k, m, o, s, u, w, y = 0;
a, c, e, g, i, r, t (independently selected) = 0 or 1;
n = 1; j, l, v, x (independently selected) = 0 or 1;
R = PEG.
```

#### **FIG. 40C**

Yeast expressed tPA B = A; i = 0.

- 1. endoglycanase
- 2. Galactosyltransferase, UDP-Gal-PEG

A = B; a-n, r-y = 0; o = 1; R' = Gal-PEG.

# FIG. 40D

Insect or fungi cell expressed tPA A = B; a-d, f, h, j-o, s, u, v-y = 0; e, g, i, n, r, t (independently selected) = 0 or 1.

- 1. alpha and beta mannosidases
- 2. Galactosyltransferase, UDP-Gal-PEG

A = B; a-n, r-y = 0; o = 1; R' = Gal-PEG.

# FIG. 40E

```
Insect or fungi cell expressed tPA

A = B; a-d, f, h, j-o, s, u, v-y = 0;
e, g, i, n, r, t (independently selected) = 0 or 1.
```

- 1. GNT's 1&2, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal-PEG

```
A = B; b, d, f, h, j-o, s, u, w, y = 0;
a, c, e, g, i, r, t, v, x (independently selected)= 0 or 1;
n = 1; R = PEG.
```

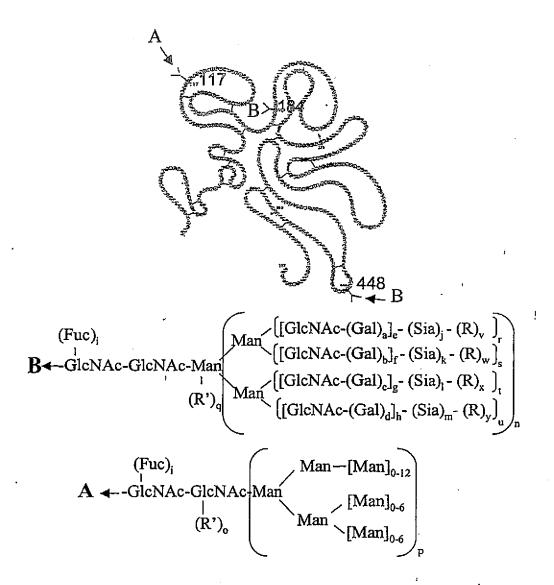
# FIG. 40F

```
Insect or fungi cell expressed tPA
A = B; a-d, f, h, j-o, s, u, v-y = 0;
e, g, i, n, r, t (independently selected) = 0 or 1.
```

- 1. GNT's 1 & 2, UDP-GlcNAc
- 2. Galactosidase (synthetic enzyme), PEG-Gal-F.

```
A = B; b, d, f, h, j-o, s, u, w, y = 0;
a, c, e, g, i, r, t, v, x (independently selected)= 0 or 1;
n = 1; R = PEG.
```

# FIG. 40G



a-d, i, n-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 4. j-m (independently selected) = 0 to 20. R = polymer; R' = sugar, glycoconjugate.

FIG. 40H

```
NSO expressed tPA
A = B; a-m, r-u (independently selected) = 0 or 1;
n = 1; o, p, q, v-y = 0
```

- 1. sialidase, alpha-galactosidase
- 2. CMP-SA-levulinate, ST3Gal3,
- 3. H<sub>4</sub>N<sub>2</sub>-PEG

```
A = B; a-m, r-y (independently selected) = 0 or 1;

n = 1; o, p, q = 0;

v-y (independently selected) = 1,

when j-m (independently selected) is 1;

R = PEG.
```

### FIG. 401

```
CHO, BHK, 293 cells, Vero expressed tPA a-g, n, p = 1; h = 1 to 3; j-m, i, (independently selected) = 0 or 1; r-u (independently selected) = 0 to 1; q, o, v-y = 0.
```

- 1. alpha and beta Mannosidases
- 2. CMP-SA, ST3Gal3
- 3. Galactosyltransferase, UDP-Gal-PEG

```
a-g, n = 1; h = 1 to 3;
i, r-u (independently selected) = 0 or 1; o = 1;
q, p, v-y = 0; j-m (independently selected) = 0 or 1;
R' = Gal-PEG
```

#### FIG. 40J

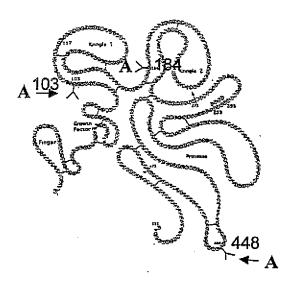
```
Plant expressed tPA

A = B; a-d, f, h, j-m, s, u, v-y = 0;
e, g, i, q, r, t (independently selected) = 0 or 1;
n = 1; R' = xylose
```

- 1. hexosaminidase,
- 2. alpha mannosidase and xylosidase
- 3. GlcNAc transferase, UDP-GlcNAc-PEG

```
A = B; a-d, f, h, j-n, s, u, v-y = 0;
e, g, i, r, t (independently selected) = 0;
q = 1; R' = GlcNAc-PEG.
```

FIG. 40K



$$\mathbf{A} \leftarrow \begin{bmatrix} \left[ \operatorname{GlcNAc-(Gal)}_{a} \right]_{e^{-}} \left( \operatorname{Sia} \right)_{j^{-}} \left( \operatorname{Rl}_{v} \right)_{r} \\ \left[ \left[ \operatorname{GlcNAc-(Gal)}_{b} \right]_{f^{-}} \left( \operatorname{Sia} \right)_{k^{-}} \left( \operatorname{Rl}_{w} \right)_{s} \\ \left[ \left[ \operatorname{GlcNAc-(Gal)}_{d} \right]_{g^{-}} \left( \operatorname{Sia} \right)_{t^{-}} \left( \operatorname{Rl}_{v} \right)_{t^{-}} \\ \left[ \left[ \operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left( \operatorname{Sia} \right)_{m^{-}} \left( \operatorname{Rl}_{v} \right)_{y} \right]_{u} \\ q = \begin{bmatrix} \left[ \operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left( \operatorname{Sia} \right)_{m^{-}} \left( \operatorname{Rl}_{v} \right)_{y} \right]_{u} \\ q = \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left( \operatorname{Sia} \right)_{m^{-}} \left( \operatorname{Rl}_{v} \right)_{y} \\ q = \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left( \operatorname{Sia} \right)_{m^{-}} \left( \operatorname{Rl}_{v} \right)_{y} \\ q = \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left( \operatorname{Sia} \right)_{m^{-}} \left( \operatorname{Rl}_{v} \right)_{y} \\ q = \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left( \operatorname{Sia} \right)_{m^{-}} \left( \operatorname{Rl}_{v} \right)_{y} \\ q = \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left( \operatorname{Sia} \right)_{m^{-}} \left( \operatorname{Rl}_{v} \right)_{y} \\ q = \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left( \operatorname{Sia} \right)_{m^{-}} \left( \operatorname{Rl}_{v} \right)_{y} \\ q = \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left( \operatorname{Sia} \right)_{y} \\ q = \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left( \operatorname{Sia} \right)_{y} \\ q = \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left( \operatorname{Sia} \right)_{y} \\ q = \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left( \operatorname{Sia} \right)_{y} \\ q = \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left( \operatorname{Sia} \right)_{y} \\ q = \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{d} \right]_{y} \\ q = \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{d} \\ q = \begin{bmatrix} \operatorname{GlcNAc-(Gal)}_{d$$

a-d, i, q-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = polymer.

FIG. 40L

CHO, BHK, 293 cells, Vero expressed TNK tPA a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

- 1. Sialidase
- 2. CMP-SA-PEG (16 mol eq), ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.
```

#### FIG. 40M

```
CHO, BHK, 293 cells, Vero expressed TNK tPA a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.
```

- 1. Sialidase
- 2. CMP-SA-PEG (1.2 mol eq), ST3Gal3
- 3. CMP-SA (16 mol eq), ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

### **FIG. 40N**

```
NSO expressed TNK tPA

a-d, i-m, q-u (independently selected) = 0 or 1;

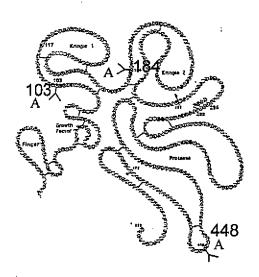
e-h = 1; v-y = 0;

Sia (independently selected) = Sia or Gal.
```

- 1. Sialidase and α-galactosidase
- 2. Galactosyltransferase, UDP-Gal

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.
```

FIG. 400



$$\mathbf{A} \leftarrow \begin{bmatrix} \left[ \operatorname{GlcNAc-(Gal)}_{a} \right]_{e^{-}} \left( \operatorname{Sia} \right)_{j} - \left( \operatorname{R} \right)_{v} \\ \left[ \left[ \operatorname{GlcNAc-(Gal)}_{b} \right]_{f^{-}} \left( \operatorname{Sia} \right)_{k} - \left( \operatorname{R} \right)_{w} \right]_{s} \\ \left[ \left[ \operatorname{GlcNAc-(Gal)}_{c} \right]_{g^{-}} \left( \operatorname{Sia} \right)_{l} - \left( \operatorname{R} \right)_{x} \right]_{t} \\ \left[ \left[ \operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left( \operatorname{Sia} \right)_{m^{-}} \left( \operatorname{R} \right)_{y} \right]_{u} \right]_{q} \\ = \underbrace{\left[ \left[ \operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left( \operatorname{Sia} \right)_{m^{-}} \left( \operatorname{R} \right)_{y} \right]_{u}}_{q} \left[ \underbrace{\left[ \operatorname{GlcNAc-(Gal)}_{d} \right]_{h^{-}} \left( \operatorname{Sia} \right)_{m^{-}} \left( \operatorname{R} \right)_{y} \right]_{u}}_{q} \right]_{q}}_{q}$$

a-d, i, q-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = polymer.

FIG. 40P

CHO, BHK, 293 cells, Vero expressed TNK tPA a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

- 1. Sialidase
- 2. CMP-SA-PEG (16 mol eq), ST3Gal3
- 3. CMP-SA, ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

# FIG. 40Q

```
CHO, BHK, 293 cells, Vero expressed TNK tPA a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.
```

```
    CMP-SA-levulinate, ST3Gal3,
buffer, salt
    H<sub>4</sub>N<sub>2</sub>-PEG
```

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

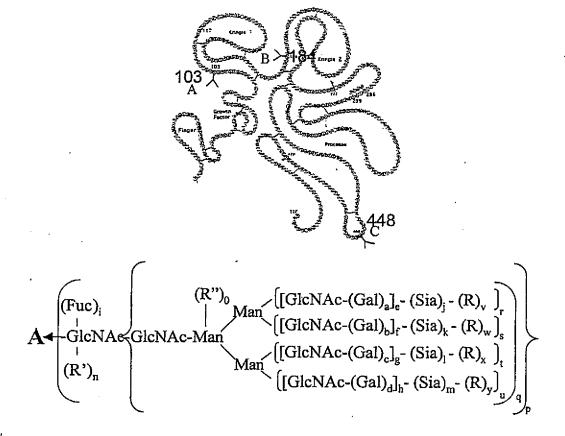
## FIG. 40R

CHO, BHK, 293 cells, Vero expressed TNK tPA a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.

1. CMP-SA, α2,8-ST

a-d, i, q-u (independently selected) = 0 or 1; e-h = 1; j-m (independently selected) = 0-20; v-y (independently selected) = 0.

FIG. 40S



a-d, i, n-y (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 6.

j-m (independently selected) = 0 to 100.

R = modifying group, mannose, oligo-mannose;

R' = H, glycosyl residue, modifying group, glycoconjugate.

R" = glycosyl residue.

FIG. 40T

```
Insect cell expressed TNK tPA
a-d, f, h, j-m, s, u, v-y = 0;
e, g, i, q, r, t (independently selected) = 0 or 1.
```

- 1. GNT's 1,2,4,5, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal-PEG

```
a-i, q-u (independently selected) = 0 or 1;

j-m = 0; v-y (independently selected) = 1,

when e-h (independently selected) is 1;

R = PEG.
```

# FIG. 40U

```
Yeast expressed TNK tPA
a-m = 0; q-y (independently selected) = 0 to 1; p = 1;
R (branched or linear) = Man, oligomannose.
```

- 1. Endoglycanase
- 2. Galactosyltransferase, UDP-Gal-PEG

```
a-m, p-y = 0; n (independently selected) = 0 or 1; R' = -Gal-PEG.
```

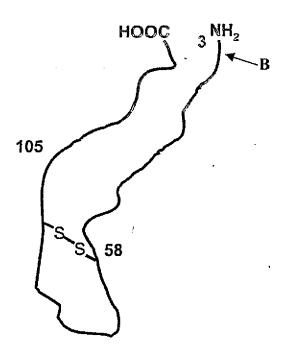
# **FIG. 40V**

CHO, BHK, 293 cells, Vero expressed TNK tPA a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

- 1. CMP-SA-linker-Gal-UDP, ST3Gal3
- 2. Galactosyltransferase, anti-TNF IG chimera produced in CHO.

a-m, r-u (independently selected) = 0 or 1; p, q = 1; n = 0; v-y (independently selected) = 0 or 1; R = linker-anti-TNF IG chimera protein.

FIG. 40W



$$\mathbf{B} \leftarrow \begin{bmatrix} (\mathrm{Sia})_{b} \\ -\mathrm{GalNAc-(Gal)_{a}-(Sia)_{c}-(R)_{d}} \end{bmatrix}_{c}$$

a-c, e (independently selected) = 0 or 1; d = 0; R = modifying group, mannose, oligomannose.

FIG. 41A

CHO, BHK, 293 cells, Vero expressed IL-2 a-c, e (independently selected) = 0 or 1; d = 0

- 1. Sialidase
- 2. CMP-SA-PEG, ST3Gal1

a-d, e (independently selected) = 0 or 1; R = PEG.

# FIG. 41B

Insect cell expressed IL-2 a, e (independently selected) = 0 or 1; b, c, d = 0.

- 1. Galactosyltransferase, UDP-Gal
- 2. CMP-SA-PEG, ST3Gal1

a, c, d, e (independently selected) = 0 or 1; R = PEG.

FIG. 41C

```
E. coli expressed IL-2
a-e = 0.

1. GalNAc Transferase, UDP-GalNAc
2. CMP-SA-PEG, sialyltransferase

c, d, e (independently selected) = 0 or 1;
a, b = 0; R = PEG.
```

# FIG. 41D

```
NSO expressed IL-2
a, e (independently selected) = 0 or 1;
b, c, d = 0

1. CMP-SA-levulinate, ST3Gal1
2. H_4N_2-PEG

a, c, d, e (independently selected) = 0 or 1;
b = 0; R = PEG.
```

FIG. 41E

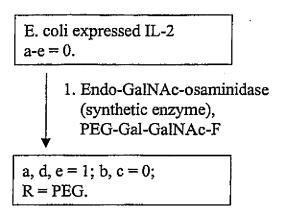


FIG. 41F

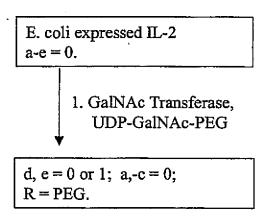


FIG. 41G

2 peptides

A and A' - N-linked sites

 ${f B}$  - O-linked sites

$$(Fuc)_{i} \qquad ([GlcNAc-(Gal)_{a}]_{e}-(Sia)_{j}-(R)_{v})_{r}$$

$$([GlcNAc-(Gal)_{b}]_{f}-(Sia)_{k}-(R)_{w})_{s}$$

$$([GlcNAc-(Gal)_{c}]_{g}-(Sia)_{l}-(R)_{x})_{t}$$

$$([GlcNAc-(Gal)_{d}]_{h}-(Sia)_{m}-(R)_{y})_{u}$$

$$([GlcNAc-(Gal)_{d}]_{h}-(Sia)_{m}-(R)_{y})_{u}$$

$$([GlcNAc-(Gal)_{d}]_{h}-(Sia)_{m}-(R)_{y})_{u}$$

$$\mathbf{B} \quad \bullet \begin{array}{c} \text{(Sia)}_{o} \\ \text{-GalNAc-(Gal)}_{n}\text{-(Sia)}_{p}\text{- (R)}_{z} \end{array} \right]_{q}$$

a-d, i, n-u (independently selected) = 0 or 1. aa, bb (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 20. v-z=0; R = polymer, glycoconjugate.

FIG. 42A

CHO, BHK, 293s cells, Vero, MDCK, HEKC expressed Factor VIII.

e-h = 1 to 4;

aa, bb, a-d, j-m, i, n-u (independently selected) = 0 or 1;

v-z = 0.

- 1. Sialidase
- 2. CMP-SA-PEG, ST3Gal3

e-h = 1 to 4; aa, bb, a-d, i, n, q-u (independently selected) = 0 or 1; o, p, z = 0; j-m, v-y (independently selected) = 0 or 1; R = PEG.

## FIG. 42B

CHO, BHK, 293S cells, Vero, MDCK, 293S, HEKC expressed Factor VIII.

e-h = 1 to 4;

aa, bb, a-d, j-m, i, n-u (independently selected) = 0 or 1;

v-z = 0.

- 1. Sialidase
  - 2. CMP-SA-PEG, ST3Gal3
  - 3. ST3Gal1, CMP-SA

e-h = 1 to 4; aa, bb, a-d, i, n, p-u (independently selected) = 0 or 1; o, z = 0; j-m, v-y (independently selected) = 0 or 1; R = PEG.

#### FIG. 42C

```
CHO, BHK, 293s cells, Vero, MDCK, HEKC expressed Factor VIII.

e-h = 1 to 4;

aa, bb, a-d, j-m, i, n-u (independently selected)=0 or 1;

v-z = 0.
```

#### 1. CMP-SA-PEG, ST3Gal3

```
e-h = 1 to 4;
aa, bb, a-d, i, n-u (independently selected) = 0 or 1;
z = 0; j-m, v-y (independently selected) = 0 or 1;
R = PEG.
```

# FIG. 42D

```
CHO, BHK, 293S cells, Vero, MDCK, HEKC expressed Factor VIII.

e-h = 1 to 4;

aa, bb, a-d, j-m, i, n-u (independently selected) 0 or 1;

v-z = 0.
```

# 1. CMP-SA-PEG, ST3Gal1

```
e-h = 1 to 4;
aa, bb, a-d, i, n-u (independently selected) = 0 or 1;
z = 0; j-m, v-y (independently selected) = 0 or 1;
R = PEG.
```

#### FIG. 42E

```
CHO, BHK, 293S cells, Vero, MDCK, HEKC expressed Factor VIII.

e-h = 1 to 4;

aa, bb, a-d, j-m, i, n-u (independently selected)=0 or 1;

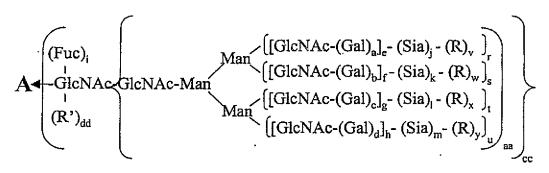
v-z = 0.
```

# 1. CMP-SA-PEG, α2,8-ST

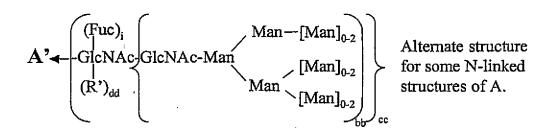
```
e-h = 1 to 4;
aa, bb, a-d, i, n-y (independently selected) = 0 or 1;
z = 0; j-m (independently selected) = 0 to 2;
v-y (independently selected) = 1,
when j-m (independently selected) is 2;
R = PEG.
```

# FIG. 42F

2 peptides
A Or A? - N-linked sites
B - O-linked sites



$$\mathbf{B} = \left( \begin{array}{c} (\mathrm{Sia})_{0} \\ -(\mathrm{GalNAc-(Gal)}_{n} - (\mathrm{Sia})_{p} - (\mathrm{R})_{z} \end{array} \right)_{0}$$



a-d, i, n-u, (independently selected) = 0 or 1.
aa, bb, cc, dd (independently selected) = 0 or 1.
e-h (independently selected) = 0 to 6.
j-m (independently selected) = 0 to 20.
v-z = 0;
R = modifying group, mannose, oligo-mannose.
R' = H, glycosyl residue, modifying group,
glycoconjugate.

FIG. 42G

```
CHO, BHK, 293S cells, Vero, MDCK, HEKC expressed Factor VIII.

e-h = 1 to 4;

aa, bb, cc, a-d, j-m, i, n-u (independently selected) = 0 or 1;

dd, v-z = 0.
```

CMP-SA-levulinate, ST3Gal3,
 L<sub>4</sub>N<sub>2</sub>-PEG

```
e-h = 1 to 4;
aa, bb, cc, a-d, i, n-u (independently selected) = 0 or 1;
dd, z = 0; j-m, v-y (independently selected) = 0 or 1;
R = PEG.
```

## FIG. 42H

```
CHO, BHK, 293S cells, Vero, MDCK, HEKC expressed Factor VIII.

e-h = 1 to 4;

aa, bb, cc, a-d, j-m, i, n-u (independently selected) = 0 or 1;

dd, v-z = 0.
```

1. endo-H2. galactosyltransferase, UDP-Gal-PEG

```
e-h = 1 to 4;
aa, bb, dd, a-d, i, j-u (independently selected) = 0 or 1;
cc, v-z = 0; R' = -Gal-PEG.
```

## FIG. 421

```
CHO, BHK, 293S cells, Vero, MDCK, HEKC expressed Factor VIII.

e-h = 1 to 4;

aa, bb, cc, a-d, j-m, i, n-u (independently selected) = 0 or 1;

dd, v-z = 0.
```

- 1. ST3Gal3, CMP-SA
- 2. endo-H
- 3. galactosyltransferase, UDP-Gal-PEG

```
e-h = 1 to 4;
aa, bb, dd, a-d, i, j-u (independently selected) = 0 or 1;
cc, v-z = 0; R' = -Gal-PEG.
```

# FIG. 42J

```
CHO, BHK, 293S cells, Vero, MDCK, HEKC expressed Factor VIII.

e-h = 1 to 4;

aa, bb, cc, a-d, j-m, i, n-u (independently selected) = 0 or 1;

dd, v-z = 0.
```

- 1. mannosidases
- 2. GNT 1 & 2, UDP-GlcNAc
- 3. galactosyltransferase, UDP-Gal-PEG

```
e-h = 1 to 4;
aa, a-d, i, j-y (independently selected) = 0 or 1;
bb, cc, dd, z = 0; R = PEG.
```

# FIG. 42K

```
CHO, BHK, 293S cells, Vero, MDCK, HEKC expressed Factor VIII.
e-h = 1 to 4;
aa, bb, cc, a-d, j-m, i, n-u (independently selected) = 0 or 1;
dd, v-z = 0.
```

- 1. mannosidases
- 2. GNT-1,2, 4 & 5; UDP-GlcNAc
- ★ 3. galactosyltransferase, UDP-Gal
  - 4. ST3Gal3, CMP-SA

```
e-h = 1 to 4;
aa, bb, cc, a-d, i, j-q (independently selected) = 0 or 1;
dd, v-z=0.
```

# FIG. 42L

```
CHO, BHK, 293S cells, Vero, MDCK, HEKC expressed Factor VIII.

e-h = 1 to 4;

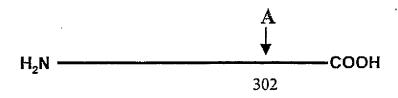
aa, bb, cc, a-d, j-m, i, n-u (independently selected) = 0 or 1;

dd, v-z = 0.
```

1. mannosidases
2. GNT-1, ÚDP-GlcNAc-PEG

```
e-h = 0 to 4;
aa, a-d, i, j-y (independently selected) = 0 or 1;
bb, cc, dd, z = 0.
```

#### FIG. 42M



$$\mathbf{A} \leftarrow \begin{bmatrix} (\operatorname{Fuc})_{i} & \operatorname{Man} & \left[ (\operatorname{GlcNAc-(Gal)}_{a})_{e^{-}} & (\operatorname{Sia})_{j} - (\operatorname{R})_{v} \right]_{r} \\ (\operatorname{GlcNAc-GlcNAc-Man} & \left[ (\operatorname{GlcNAc-(Gal)}_{b})_{f^{-}} & (\operatorname{Sia})_{k} - (\operatorname{R})_{w} \right]_{s} \\ \operatorname{Man} & \left[ (\operatorname{GlcNAc-(Gal)}_{c})_{g^{-}} & (\operatorname{Sia})_{i} - (\operatorname{R})_{x} \right]_{t} \\ \left[ (\operatorname{GlcNAc-(Gal)}_{d})_{h^{-}} & (\operatorname{Sia})_{m^{-}} & (\operatorname{R})_{y} \right]_{u} \\ \operatorname{GlcNAc-(Gal)}_{d} & \operatorname{GlcNAc-(Gal)}_{d} &$$

a-d, i, q-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = polymer.

FIG. 43A

CHO, BHK, 293 cells, Vero expressed Urokinase. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

- 1. Sialidase
- 2. CMP-SA-PEG (16 mol eq), ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.

## FIG. 43B

CHO, BHK, 293 cells, Vero expressed Urokinase. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

- 1. Sialidase
- 2. CMP-SA-PEG (1.2 mol eq), ST3Gal3
- 3. CMP-SA (16 mol eq), ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y (independently selected) = 0 or 1; R = PEG.

#### FIG. 43C

```
CHO, BHK, 293 cells, Vero expressed Urokinase. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.
```

- 1. Sialidase
- 2. CMP-SA-PEG (16 mol eq), ST3Gal3
- 3. CMP-SA, ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

#### FIG. 43D

```
CHO, BHK, 293 cells, Vero expressed Urokinase. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.
```

- 1. CMP-SA-levulinate, ST3Gal3, buffer, salt
- 2. H<sub>4</sub>N<sub>2</sub>-PEG

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

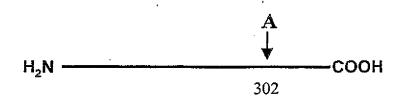
#### FIG. 43E

```
CHO, BHK, 293 cells, Vero expressed Urokinase.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.
```

1. CMP-SA,  $\alpha$ 2,8-ST

```
a-d, i, q-u (independently selected) = 0 or 1;
e-h = 1;
j-m (independently selected) = 0-20;
v-y (independently selected) = 0.
```

FIG. 43F



$$\mathbf{A} \leftarrow \begin{bmatrix} (\operatorname{Fuc})_{i} & & & \\ (\operatorname{GlcNAc-(Gal)}_{a})_{e} - (\operatorname{Sia})_{j} - (\operatorname{R})_{v} \\ (\operatorname{GlcNAc-(Gal)}_{b})_{f} - (\operatorname{Sia})_{k} - (\operatorname{R})_{w} \end{bmatrix}_{s} \\ (\operatorname{GlcNAc-(Gal)}_{b})_{g} - (\operatorname{Sia})_{l} - (\operatorname{R})_{x} \\ (\operatorname{R}')_{n} & & & & \\ (\operatorname{GlcNAc-(Gal)}_{d})_{h} - (\operatorname{Sia})_{m} - (\operatorname{R})_{y} \end{bmatrix}_{u} \\ q \\ p \\ (\operatorname{GlcNAc-(Gal)}_{d})_{h} - (\operatorname{Sia})_{m} - (\operatorname{R})_{y} \end{bmatrix}_{u}$$

a-d, i, n, p-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = modifying group, mannose, oligo-mannose; R' = H, glycosyl residue, modifying group, glycoconjugate.

FIG. 43G

```
Insect cell expressed Urokinase.
a-d, f, h, j-n, s, u, v-y = 0;
e, g, i, q, r, t (independently selected) = 0 or 1.
```

- 1. GNT's 1,2,4,5, UDP-GlcNAc
- · 2. Galactosyltransferase, UDP-Gal-PEG

```
a-i, q-u (independently selected) = 0 or 1;
j-n = 0; v-y (independently selected) = 1,
when e-h (independently selected) is 1;
R = PEG.
```

#### FIG. 43H

```
Yeast expressed Urokinase.

a-n = 0;

q-y (independently selected) = 0 to 1;

p = 1; R (branched or linear) = Man, oligomannose.
```

- 1. Endoglycanase
- 2. Galactosyltransferase, UDP-Gal
- 3. CMP-SA-PEG, ST3Gal3

```
a-m, p-y = 0; n (independently selected) = 0 or 1; R' = -Gal-Sia-PEG.
```

## FIG. 431

```
CHO, BHK, 293 cells, Vero expressed Urokinase.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; n, v-y = 0.
```

- 1. CMP-SA-linker-SA-CMP, ST3Gal3
- 2. ST3Gal1, desialylated Urokinase produced in CHO.
- 3. CMP-SA, ST3Gal3, ST3Gal1

```
a-m, q-u (independently selected) = 0 or 1;

p = 1; n = 0;

v-y (independently selected) = 0 or 1;

R = linker-Urokinase.
```

#### FIG. 43J

```
Isolated Urokinase.

a-d, i-m, q-u (independently selected) = 0 or 1;

e-h = 1; v-y = 0; n = 0;

Sia (independently selected) = Sia or SO<sub>4</sub>;

Gal (independently selected) = Gal or GalNAc;

GlcNAc (independently selected) = GlcNAc or GlcNAc-Fuc.
```

sulfohydrolase
 CMP-SA-PEG, sialyltransferase

```
a-d, i-m, q-u (independently selected) = 0 or 1;
n = 0; e-h = 1; Sia = Sia;
Gal (independently selected) = Gal or GalNAc;
GlcNAc (independently selected) = GlcNAc or GlcNAc-Fuc.
v-y (independently selected) = 0 or 1;
R = PEG.
```

## FIG. 43K

Isolated Urokinase.

a-d, i-m, q-u (independently selected) = 0 or 1;

e-h = 1; n = 0; v-y = 0;

Sia (independently selected) = Sia or SO<sub>4</sub>;

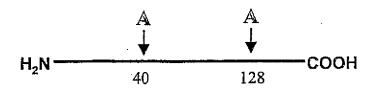
Gal (independently selected) = Gal or GalNAc;

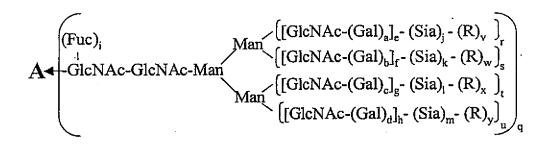
GlcNAc (independently selected) = GlcNAc or GlcNAc-Fuc.

- 1. sulfohydrolase, hexosaminidase
- 2. UDP-Gal-PEG, galactosyltransferase

a-d, i, q-u (independently selected) = 0 or 1; e-h = 1; j-n = 0; Gal (independently selected) = Gal; GlcNAc (independently selected) = GlcNAc or GlcNAc-Fuc; v-y (independently selected) = 0 or 1; R = PEG.

FIG. 43L





a-d, i, q-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = polymer, glycoconjugate.

**FIG. 44A** 

```
CHO, BHK, 293 cells, Vero expressed DNase I. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.
```

- 1. Sialidase
- 2. CMP-SA-PEG (16 mol eq), ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1;
v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.
```

## FIG. 44B

```
CHO, BHK, 293 cells, Vero expressed DNase I. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.
```

- 1. Sialidase
- 2. CMP-SA-PEG (1.2 mol eq), ST3Gal3
- 3. CMP-SA (16 mol eq), ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

#### FIG. 44C

```
CHO, BHK, 293 cells, Vero expressed DNase I. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.
```

- 1. Sialidase
- 2. CMP-SA-PEG (16 mol eq), ST3Gal3
- 3. CMP-SA, ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

#### FIG. 44D

```
CHO, BHK, 293 cells, Vero expressed DNase I. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.
```

- CMP-SA-levulinate, ST3Gal3, buffer, salt
   H<sub>4</sub>N<sub>2</sub>-PEG
- a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y (independently selected) = 0 or 1; R = PEG.

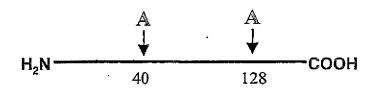
#### FIG. 44E

CHO, BHK, 293 cells, Vero expressed DNase I. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.

1. CMP-SA, α2,8-ST

a-d, i, q-u (independently selected) = 0 or 1; e-h = 1; j-m (independently selected) = 0-20; v-y (independently selected) = 0.

## FIG. 44F



$$\mathbf{A} \leftarrow \begin{bmatrix} (\operatorname{Fuc})_{i} & & & \\ (\operatorname{Fuc})_{i} & & & \\ (\operatorname{GlcNAc-(Gal)}_{a}]_{e^{-}} (\operatorname{Sia})_{j^{-}} (\operatorname{R})_{v} \\ (\operatorname{GlcNAc-(Gal)}_{b}]_{f^{-}} (\operatorname{Sia})_{k^{-}} (\operatorname{R})_{w} \\ (\operatorname{R}')_{n} & & & \\ (\operatorname{GlcNAc-(Gal)}_{d}]_{g^{-}} (\operatorname{Sia})_{i^{-}} (\operatorname{R})_{x} \\ (\operatorname{GlcNAc-(Gal)}_{d}]_{h^{-}} (\operatorname{Sia})_{m^{-}} (\operatorname{R})_{y} \end{bmatrix}_{u}$$

a-d, i, n, p-u (independently selected) = 0 or 1.
e-h (independently selected) = 0 to 6.
j-m (independently selected) = 0 to 100.
v-y = 0;
R = modifying group, mannose, oligo-mannose;
R' = H, glycosyl residue, modifying group,
glycoconjugate.

FIG. 44G

```
Insect cell expressed DNase I.
a-d, f, h, j-n, s, u, v-y = 0;
e, g, i, q, r, t (independently selected) = 0 or 1.
```

- 1. GNT's 1,2,4,5, UDP-GlcNAc
- 2. Galactosyltransferase, UDP-Gal-PEG

```
a-i, q-u (independently selected) = 0 or 1; j-n = 0;
v-y (independently selected) = 1,
when e-h (independently selected) is 1;
R = PEG.
```

#### FIG. 44H

```
Yeast expressed DNase I.

a-n = 0;

q-y (independently selected) = 0 to 1;

p = 1; R (branched or linear) = Man, oligomannose.
```

- 1. Endoglycanase
- 2. Galactosyltransferase, UDP-Gal
- 3. CMP-SA-PEG, ST3Gal3

a-n, p-y = 0; n (independently selected) = 0 or 1; R' = -Gal-Sia-PEG.

#### FIG. 441

CHO, BHK, 293 cells, Vero expressed DNase I. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; n, v-y=0.

- 1. CMP-SA-linker-SA-CMP, ST3Gal3
- 2. ST3Gal1, desialylated alpha-1-Proteinase inhibitor.
- 3. CMP-SA, ST3Gal3, ST3Gal1

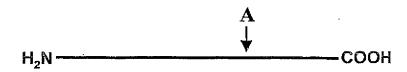
```
a-m, q-u (independently selected) = 0 or 1;

p = 1; n = 0;

v-y (independently selected) = 0 or 1;

R = linker- alpha-1-Proteinase inhibitor.
```

FIG. 44J



$$(Fuc)_{i} \\ A \leftarrow GlcNAc-Gal_{a}l_{e} - (Sia)_{j} - (R)_{v} \\ GlcNAc-Man \\ [GlcNAc-(Gal)_{b}l_{f} - (Sia)_{k} - (R)_{w}]_{s} \\ (R')_{n} \\ (R')_{n} \\ (GlcNAc-(Gal)_{c}l_{g} - (Sia)_{l} - (R)_{x} \\ [GlcNAc-(Gal)_{d}l_{h} - (Sia)_{m} - (R)_{y}]_{u} \\ [GlcNAc-(Gal)_{d}l_{h} - (Sia)_{m} - (R)_{y}]_{u} \\ (R')_{n} \\ (R')_{n}$$

a-d, i, r-u (independently selected) = 0 or 1.
e-h (independently selected) = 0 to 4.
j-m (independently selected) = 0 or 1.
n, v-y = 0; z = 0 or 1;
R = modifying group, mannose, oligo-mannose;
R' = H, glycosyl residue, modifying group,
glycoconjugate.

FIG. 45A

```
CHO, BHK, 293 cells, Vero expressed Insulin.
a-m, r-u (independently selected) = 0 or 1;
n = 0; v-y = 0; z = 1.
```

- 1. Sialidase
- 2. CMP-SA-PEG, ST3Gal3

```
a-m, r-u (independently selected) = 0 or 1;
v-y (independently selected) = 1,
when j-m (independently selected) is 1;
n = 0; R = PEG; z = 1.
```

#### FIG. 45B

```
Insect cell expressed Insulin.
a-h, j-n, s-y = 0;
i, r (independently selected) = 0 or 1; z = 1.
```

1. GNT's 1&2, UDP-GlcNAc-PEG

```
a-d, f, h, j-n, s, u, w, y = 0;
e, g, i, r, t, v, x (independently selected) = 0 or 1;
v, x (independently selected) = 1,
when e, g (independently selected) is 1;
z = 1; R = PEG.
```

## FIG. 45C

Yeast expressed Insulin.

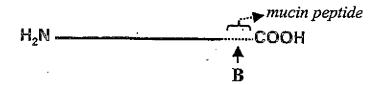
a-n=0; r-y (independently selected) = 0 to 1; z=1;

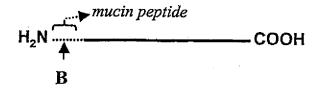
R (branched or linear) = Man, oligomannose or polysaccharide.

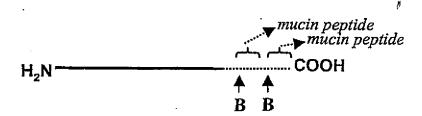
- 1. Endo-H
- 2. Galactosyltransferase, UDP-Gal-PEG

a-m, r-z= 0; n = 1; R' = -Gal-PEG.

FIG. 45D







$$\mathbf{B} = \left( \frac{(\operatorname{Sia})_{b}}{\operatorname{GalNAc-(Gal)}_{a} - (\operatorname{Sia})_{c} - (\operatorname{R})_{d}} \right)_{c}$$

a-c, e (independently selected) = 0 or 1; d = 0; R = polymer

FIG. 45E

CHO, BHK, 293 cells, Vero expressed insulinmucin fusion protein.

a-c, e (independently selected) = 0 or 1; d = 0

- 1. Sialidase
- 2. CMP-SA-PEG, ST3Gal1

a-d, e (independently selected) = 0 or 1; R = PEG.

## FIG. 45F

Insect cell expressed Insulin-mucin fusion protein. a, e (independently selected) = 0 or 1; b, c, d = 0.

1. Galactosyltransferase, UDP-Gal-PEG

a, d, e (independently selected) = 0 or 1; b, c = 0; R = PEG.

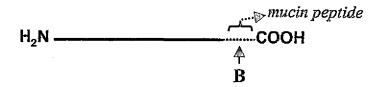
## FIG. 45G

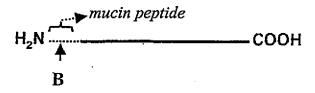
E. coli expressed Insulin-mucin fusion protein. a-e=0.

- 1. GalNAc Transferase, UDP-GalNAc
- 2. CMP-SA-PEG, sialyltransferase

c, d, e (independently selected) = 0 or 1; a, b = 0; R = PEG.

FIG. 45H





$$\mathbf{B} \leftarrow \begin{pmatrix} (\operatorname{Sia})_{b} \\ -\operatorname{GalNAc-(Gal)}_{a} - (\operatorname{Sia})_{c} - (R)_{d} \end{pmatrix}_{e}$$

$$\mathbf{C} \leftarrow (\mathbf{R}')_{\mathbf{n}}$$

a-c, e (independently selected) = 0 or 1; d = 0; R = modifying group, mannose,oligo-mannose.

FIG. 451

E. coli expressed Insulin-mucin fusion protein. a-e, n=0.

1. GalNAc Transferase, UDP-GalNAc-PEG

d, e (independently selected) = 0 or 1; a-c, n = 0; R = PEG.

## FIG. 45J

E. coli expressed Insulin-mucin fusion protein. a-e, n = 0.

- GalNAc Transferase, UDP-GalNAc-linker-SA-CMP
- 2. ST3Gal3, asialo-transferrin
- 3. CMP-SA, ST3Gal3

d, e (independently selected) = 0 or 1; a-c, n = 0; R = linker-transferrin.

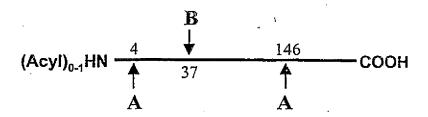
#### FIG. 45K

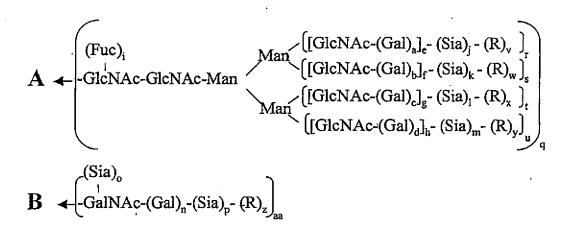
E. coli expressed Insulin (N)—no mucin peptide. a-e, n=0.

- 1. NHS-CO-linker-SA-CMP
- 2. ST3Gal3, asialo-transferrin
- 3. CMP-SA, ST3Gal3

a-e = 0; n = 1; R' = linker-transferrin.

FIG. 45L





a-d, i, n-u, aa (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = polymer, glycoconjugate.

FIG. 46A

CHO, BHK, 293 cells, Vero expressed M-antigen. a-d, i-m, o-u, aa (independently selected) = 0 or 1; n, e-h = 1; v-z=0.

- 1. Sialidase
- 2. CMP-SA-linker-lipid-A, ST3Gal3

```
a-d, i-m, q-u, aa (independently selected) = 0 or 1;
o, p, z = 0; n, e-h = 1;
v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = linker-lipid-A.
```

#### FIG. 46B

CHO, BHK, 293 cells, Vero expressed M-antigen. a-d, i-m, o-u, as (independently selected) = 0 or 1; n, e-h = 1; v-z = 0.

- 1. sialidase
- 2. CMP-SA-linker-tetanus toxin, ST3Gal1
- 3. CMP-SA, ST3Gal3

a-d, i-m, p-u, z, as (independently selected) = 0 or 1; o, v-y=0; n, e-h=1; R= tetanus toxin.

#### FIG. 46C

```
NSO expressed M-antigen.
a-d, i-n, o-u, aa (independently selected) = 0 or 1;
e-h = 1; v-z = 0;
Sia (independently selected) = Sia or Gal.
```

- 1. α-galactosidase
- 2. CMP-SA, ST3Gal3
- 2. CMP-SA-KLH, ST3Gal1

```
a-d, i-n, p-u, z, as (independently selected) = 0 or 1;
e-h = 1; o, v-y = 0;
z = 1, when p = 1;
R = KLH.
```

#### **FIG. 46D**

```
Yeast expressed M-antigen.

a-p, z = 0; q-y, aa (independently selected) = 0 to 1;

R (branched or linear) = Man, oligomannose;

GalNAc = Man.
```

α1,2-mannosidase
 GNT 1,
 UDP-GlcNAc-linker-diphtheria toxin.

e, q, l, m, r, t, u, v, aa (independently selected) =0 or 1; a-d, f-h, j, k, n-p, s, w-z = 0; Sia = Man; R = linker-diphtheria toxin.

## FIG. 46E

CHO, BHK, 293 cells, Vero expressed M-antigen. a-d, i-m, o-u, aa (independently selected) = 0 or 1; n, e-h = 1; v-z=0.

- 1. CMP-SA-levulinate, ST3Gal3,
- 2. H<sub>4</sub>N<sub>2</sub>-linker-DNA

a-d, i-m, o-y, as (independently selected) = 0 or 1; z = 0; n, e-h = 1; R = linker-DNA.

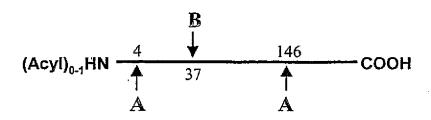
## FIG. 46F

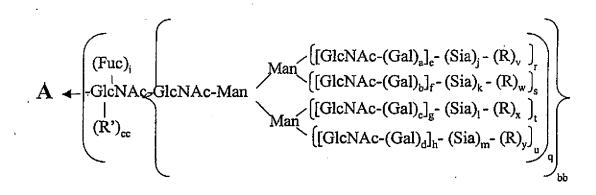
CHO, BHK, 293 cells, Vero expressed M-antigen. a-d, i-n, o-u, aa (independently selected) = 0 or 1; e-h = 1; v-z = 0.

1. CMP-SA, poly-α2,8-ST

a-d, i, n-u, aa (independently selected) = 0 or 1; e-h = 1; j-m (independently selected) = 0-100; v-z (independently selected) = 0.

#### FIG. 46G





$$\mathbf{B} \leftarrow \begin{bmatrix} (\mathrm{Sia})_{o} \\ -\mathrm{GalNAc-(Gal)}_{n} - (\mathrm{Sia})_{p} - (\mathrm{R})_{z} \end{bmatrix}_{aa}$$

a-d, i, n, q-u, aa, bb, (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-p (independently selected) = 0 to 100. Cc, v-y = 0; R = modifying group, mannose, oligo-mannose. R'= H, glycosyl residue, modifying group, glycoconjugate.

## FIG. 46H

```
Insect cell expressed M-antigen.
a-d, f, h, j-m, o, p, s, u, v-z, cc = 0;
bb = 1;
e, g, i, n, q, r, t, aa (independently selected) = 0 or 1.
```

 GNT-2, UDP-GlcNAc-linker-Neisseria protein

```
a, c, e, g, i, n, q, r, t, v, x, aa (independently selected) = 0 or 1;
b, d, f, h, j-p, s, u, w, y, z, cc = 0;
bb = 1; R = -linker-Neisseria protein.
```

#### FIG. 46!

```
Yeast expressed M-antigen.

a-p, z, cc = 0;

q-y, aa (independently selected) = 0 to 1;

bb = 1; R (branched or linear) = Man, oligomannose;

GalNAc = Man.
```

1. Endoglycanase

2. Galactosyltransferase, UDP-Gal-linker-Neisseria protein

```
a-p, r-z, bb = 0;
q, aa, cc (independently selected) = 0 or 1;
R' = -Gal-linker-Neisseria protein.
```

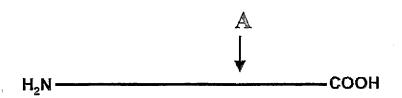
#### FIG. 46J

```
Yeast expressed M-antigen.
a-p, z, cc = 0;
q-y, aa (independently selected) = 0 to 1; bb = 1;
R (branched or linear) = Man, oligomannose;
GalNAc = Man.
```

- 1. mannosidases
- 2. GNT 1 & 2, UDP-GlcNAc
- 3. UDP-Gal, Galactosyltransferase,
- 4. CMP-SA, sialyltransferase

a, c, e, g, j, l, q, r, t, as (independently selected) = 0 or 1; b, d, f, h, k, m-p, s, u-z, cc = 0; bb = 1.

FIG. 46K



$$(Fuc)_{i} \\ A \leftarrow GlcNAc - GlcNAc - Man \\ \begin{bmatrix} [GlcNAc - (Gal)_{a}]_{e} - (Sia)_{j} - (R)_{v} \end{bmatrix}_{r} \\ [GlcNAc - (Gal)_{b}]_{f} - (Sia)_{k} - (R)_{w} \end{bmatrix}_{s} \\ (R')_{n} \\ \end{bmatrix}_{t} \\ \begin{bmatrix} [GlcNAc - (Gal)_{c}]_{g} - (Sia)_{l} - (R)_{x} \end{bmatrix}_{t} \\ \begin{bmatrix} [GlcNAc - (Gal)_{d}]_{h} - (Sia)_{m} - (R)_{y} \end{bmatrix}_{u} \\ \end{bmatrix}_{t} \\$$

a-d, i, r-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 4. j-m (independently selected) = 0 or 1. n, v-y = 0; z = 0 or 1; R = modifying group, mannose, oligo-mannose; R' = H, glycosyl residue, modifying group, glycoconjugate.

FIG. 47A

CHO, BHK, 293 cells, Vero expressed Growth Hormone.

a-m, r-u (independently selected) = 0 or 1; n = 0; v-y = 0; z = 1.

- 1. Sialidase
- 2. CMP-SA-PEG, ST3Gal3

a-m, r-u (independently selected) = 0 or 1; v-y (independently selected) = 1, when j-m (independently selected) is 1; n = 0; R = PEG; z = 1.

#### FIG. 47B

```
Insect cell expressed growth hormone.
a-h, j-n, s-y = 0;
i, r (independently selected) = 0 or 1; z = 1.
```

I. GNT's 1&2, UDP-GlcNAc-PEG

```
a-d, f, h, j-n, s, u, w, y = 0;
e, g, i, r, t, v, x (independently selected)= 0 or 1;
v, x (independently selected) = 1,
when e, g (independently selected) is 1;
z = 1; R = PEG.
```

#### FIG. 47C

Yeast expressed growth hormone.

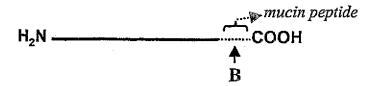
a-n = 0; r-y (independently selected) = 0 to 1; z = 1;

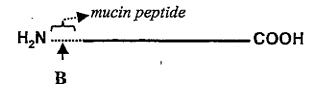
R (branched or linear) = Man, oligomannose or polysaccharide.

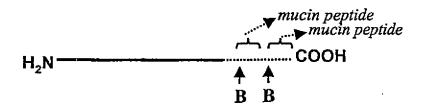
- I. Endo-H
- 2. Galactosyltransferase, UDP-Gal-PEG

a-m, r-z= 0; n = 1; R' = -Gal-PEG.

FIG. 47D







$$\mathbf{B} \leftarrow \begin{bmatrix} (\mathrm{Sia})_b \\ -\mathrm{GalNAc} - (\mathrm{Gal})_a - (\mathrm{Sia})_c - (\mathrm{R})_d \end{bmatrix}_e$$

a-c, e (independently selected) = 0 or 1;
d = 0;
R = modifying group, mannose, oligomannose.

FIG. 47E

CHO, BHK, 293 cells, Vero expressed growth hormone-mucin fusion protein. a-c, e (independently selected) = 0 or 1; d = 0

I. Sialidase

2. CMP-SA-PEG, ST3Gal1

a-d, e (independently selected) = 0 or 1; R = PEG.

#### FIG. 47F

Insect cell expressed Growth Hormone-mucin fusion protein.

a, e (independently selected) = 0 or 1; b, c, d = 0.

1. Galactosyltransferase, UDP-Gal-PEG

a, d, e (independently selected) = 0 or 1; b, c = 0; R = PEG.

## FIG. 47G

E. coli expressed growth hormone-mucin fusion protein.

a-e = 0.

- 1. GalNAc Transferase, UDP-GalNAc
- 2. CMP-SA-PEG, sialyltransferase

c, d, e (independently selected) = 0 or 1; a, b = 0; R = PEG.

#### FIG. 47H

E. coli expressed growth hormone-mucin fusion protein.

a-e, n=0.

1. GalNAc Transferase, UDP-GalNAc-PEG

d, e (independently selected) = 0 or 1; a-c, n = 0; R = PEG.

## FIG. 471

E. coli expressed growth hormone-mucin fusion protein.

a-e, n = 0.

- 1. GalNAc Transferase, UDP-GalNAc-linker-SA-CMP
- 2. ST3Gal3, asialo-transferrin
- ▼ 3. CMP-SA, ST3Gal3

d, e (independently selected) = 0 or 1; a-c, n = 0; R = linker-transferrin.

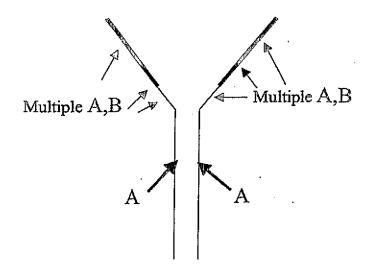
#### FIG. 47J

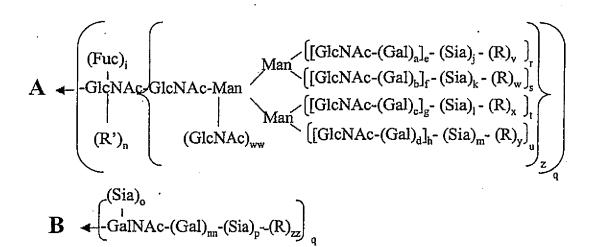
E. coli expressed growth hormone(N)—no mucin peptide.a-e, n = 0.

- 1. NHS-CO-linker-SA-CMP
- 2. ST3Gal3, asialo-transferrin
- 3. CMP-SA, ST3Gal3

a-e=0; n=1; R' = linker-transferrin.

FIG. 47K





a-d, i-m, q-u, w, z, nn, ww, zz (independently selected) = 0 or 1. e-h (independently selected) = 0 to 4.

n, v-y = 0;

R = modifying group, mannose, oligo-mannose;

R' = H, glycosyl residue, modifying group, glycoconjugate.

FIG. 48A

CHO, BHK, 293 cells, Vero or transgenic animals expressed TNF Receptor IgG Fusion.

a-m, o-u, aa (independently selected) = 0 or 1;

n = 1; v-z = 0.

- 1. CMP-SA, ST3Gal1
- 2. galactosyltransferase, UPD-Gal
- 3. CMP-SA-PEG, ST3Gal3

a-m, o-u, v-y, as (independently selected) = 0 or 1; n = 1; z = 0; R = PEG.

#### FIG. 48B

CHO, BHK, 293 cells, Vero expressed
TNF Receptor IgG Fusion.
a-m, o-u, aa (independently selected) = 0 or 1;
n = 1; v-z = 0.

1. sialidase
2. CMP-SA-PEG, ST3Gal1

a-i, p-u, z, aa (independently selected) = 0 or 1; n = 1; o, j-m, v-y = 0; R = PEG.

#### FIG. 48C

CHO, BHK, 293 cells, Vero expressed
TNF Receptor IgG Fusion.
a-m, o-u, aa (independently selected) = 0 or 1;
n = 1; v-z = 0.

 ${\tt 1.\ galactosyltransferase,\ UPD\text{-}Gal\text{-}PEG}$ 

a-m, o-u, v-y, as (independently selected) = 0 or 1; n = 1; z = 0; R = PEG.

#### FIG. 48D

CHO, BHK, 293 cells, Vero or transgenic animals expressed TNF Receptor IgG Fusion.

a-m, o-u, aa (independently selected) = 0 or 1;

n = 1; v-z = 0.

1. CMP-SA, ST3Gall
2. galactosyltransferase, UPD-Gal-PEG

a-m, o-u, v-y, aa (independently selected) = 0 or 1; n = 1; z = 0; R = PEG.

#### FIG. 48E

CHO, BHK, 293 cells, Vero or transgenic animals expressed TNF Receptor IgG Fusion.

a-m, o-u, aa (independently selected) = 0 or 1;

n = 1; v-z = 0.

1. CMP-SA-levulinate, ST3Gal12. H<sub>4</sub>N<sub>2</sub>-PEG

a-m, o-u, v-y, as (independently selected) = 0 or 1; n = 1; z = 0; R = PEG.

## FIG. 48F

CHO, BHK, 293 cells, Vero expressed
TNF Receptor IgG Fusion.
a-m, o-u, aa (independently selected) = 0 or 1;
n = 1; v-z = 0.

1. CMP-SA-PEG,  $\alpha$ 2,8-ST

a-i, o, q-u, v-z, aa (independently selected) = 0 or 1; n = 1; j-m, p (independently selected) = 0 to 2; v-z (independently selected) = 1, when j-m, p (independently selected) is 2; R = PEG.

#### FIG. 48G